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Removal of Lignin from Partially Delignified Softwoods by Soft Rot- and White Rot Fungi

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Abstract——The pattern of lignin removal from partially delignified woods by a soft rot fungus (*Chaetomium globosum* KUNZE) and a white rot fungus (*Coriolus versicolor* QUÉL.) has been studied on two softwoods (*Pinus densiflora* SIEB. et ZUCC. and *Cryptomeria japonica* D. DON) and one hardwood (*Fagus crenata* BLUME), with reference to the different acceleration pattern of wood decay caused by the partial delignification. The rapid and shorter acceleration, which was observed for the cases of *P. densiflora* and *C. japonica* attacked by *Ch. globosum* and *Co. versicolor*, respectively, was accompanied with the rapid rate of lignin removal and the higher ratio of lignin loss to weight loss at smaller extent of delignification. The slow and longer acceleration, for the cases of *P. densiflora* and *C. japonica* attacked by *Co. versicolor* and *Ch. globosum*, respectively, was accompanied with the slow or poor overall removal of lignin. In the former case, the lignin was removed apparently at slower rate than non-lignin components, and removal or modification of lignin probably acts largely for facilitating fungal enzyme systems to gain access to the carbohydrates. In the latter case, the ratio of lignin loss to weight loss increased in proportion to the extent of delignification and reached the maximum level at the moderate extent of delignification. In the case of *F. crenata* which has high susceptibility to both fungi, the rate of lignin removal and the ratio of lignin loss to weight loss were always slower and smaller in *Ch. globosum* than in *Co. versicolor*.

Introduction

Soft rot- and white rot fungi have a limited or lower capacity to attack softwoods containing higher amount of lignin than hardwoods. Acceleration of attacking capacity of soft rotters on softwoods caused by partial delignification was reported by several authors[1,2,3]. Such an accelerative effect was also observed for a white rot fungus (*Coriolus versicolor*) by TAKAHASHI and NISHIMOTO[3].

Lignin-degrading ability of white rotters is widely known and regarded recently by some investigators as an effective means to gain access to the cellulose in lignified cell wall[4,5]. According to this theory, removal of lignin from cell wall may facilitate the action of cellulolytic enzyme system in the attack of softwoods by white rot fungi. In the case of soft rot fungi, having a lower capacity to attack softwoods and degrade lignin in wood, lignin removal should be more effective than that of white rotters. Higher acceleration was observed expectedly in two softwoods for a soft rot fungus used, but the pattern of acceleration considerably varied with wood species[3].

In the present investigation, the pattern of lignin removal from partially de-

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lignified woods by a soft rot fungus (*Chaetomium globosum* KUNZE) and a white rot fungus (*Coriolus versicolor* QUÉL.) has been studied on two softwoods (*Pinus densiflora* SIEB. et ZUCC. and *Cryptomeria japonica* D. DON) and one hardwood (*Fagus crenata* BLUME), with reference to the different acceleration pattern of wood decay described in the previous report[3].

Materials and Methods

Preparation of samples

Wood blocks, 2.0 (tangential) × 2.0 (radial) × 0.5 (longitudinal) cm, were subjected to the chlorite treatment and fungal attack[3]. The decayed blocks were separated into six to ten groups according to weight losses caused by chlorite treatment before decay. Weights of each group after each of three treatments (extraction with ethanol-benzene, delignification and exposure to fungal attack) were calculated from the weights of each block determined after each treatment. Each group was separately ground to pass a 40-mesh sieve and throughly air dried.

Lignin determination

The Klason lignin content was determined by the JIS P 8008-1961. The acid-soluble lignin content was determined on the hydrolysate from the Klason lignin by measuring ultraviolet absorption at 205 nm in 1 cm quartz cells using a Shimadzu MPS-50 spectrophotometer. The acid-soluble lignin content was calculated according to the following equation[3,6,7]:

$$\% \text{ acid-soluble lignin} = \frac{(A_s - A_b) \times V}{110 \times W} \times 100$$

where A_s is the absorbance of the sample, A_b is the absorbance of the blank, W is the weight of the sample in g, and V is the volume in litres of the solution. The total lignin content was calculated as insoluble Klason lignin plus the acid-soluble lignin estimated spectrophotometrically.

Results and Discussion

Table 1 shows the loss of weight by chlorite treatment and fungal attack of each group for lignin analysis. As reported previously[3], rapid and shorter acceleration of fungal attack chracterizes the cases of *Pinus densiflora* attacked by *Chaetomium globosum* and *Cryptomeria japonica* by *Coriolus versicolor*. Slow and longer acceleration characterizes *P. densiflola* attacked by *Co. versicolor* and *C. japonica* by *Ch. globosum*. However, in the case of *Fagus crenata*, acceleration was not greater than in the two softwoods, but was rather slow and longer.

Figs. 1, 2 and 3 show the results for the loss of lignin from each group of the three wood species subjected to chlorite treatment and fungal attack. Each point

Table 1. The loss of weight in the samples for lignin analysis caused by chlorite treatment and exposure to fungal attack.

Fungus \ Wood	*Pinus densiflora*		*Cryptomeria japonica*		*Fagus crenata*	
	Weight loss by chlorite treatment (%)	Weight loss by decay (%)	Weight loss by chlorite treatment (%)	Weight loss by decay (%)	Weight loss by chlorite treatment (%)	Weight loss by decay (%)
Chaetomium globosum	0*	3.50	0*	0.00	0*	36.50
	0.46	24.11	2.53	3.83	2.52	40.45
	3.56	34.74	4.50	13.67	3.75	46.70
	6.48	36.35	5.28	16.31	10.64	40.62
	8.70	39.24	7.21	22.93	14.02	41.44
	13.61	43.66	7.96	32.21	17.81	54.81
	15.41	43.78	11.37	34.35		
	18.37	43.64	13.88	45.91		
	20.29	40.89	16.05	50.53		
Coriolus versicolor	0*	14.00	0*	11.50	0*	45.50
	2.59	18.61	3.48	41.67	4.17	42.70
	3.56	19.91	4.21	41.32	5.40	45.78
	7.58	24.68	7.35	37.20	6.56	48.71
	8.49	28.82	10.26	41.23	10.77	56.75
	9.45	29.65	10.79	39.67	13.48	52.58
	12.35	40.91	11.31	42.17	15.26	63.97
	15.09	36.39	11.85	40.37		
	17.00	39.35	12.92	42.92		
	18.06	32.28	15.06	37.45		

All values are expressed on the basis of the weight of extractive-free wood before chlorite treatment. 0*; Extracted with ethanol-benzene, not treated with sodium chlorite and acetic acid.

Table 2. Klason and acid-soluble lignin contents of sound woods*.

Wood	Lignin content (%)		
	Klason	Acid-soluble	Total
Pinus densiflora	28.33	0.26	28.59
Cryptomeria japonica	33.67	0.37	34.04
Fagus crenata	26.47	2.14	28.61

* An average of the values from three separate Klason hydrolyses of 0.3 mm thick wood shavings[3].

is an average of the data from two separate hydrolyses. Percent loss of lignin is expressed on the basis of the original amount of lignin in sound wood shown in Table 2. Dotted line on each figure represents the pattern of lignin removal from 0.3 mm thick shavings of each wood species during chlorite treatment[3]. Comparing the pattern of lignin removal during chlorite treatment for different forms of

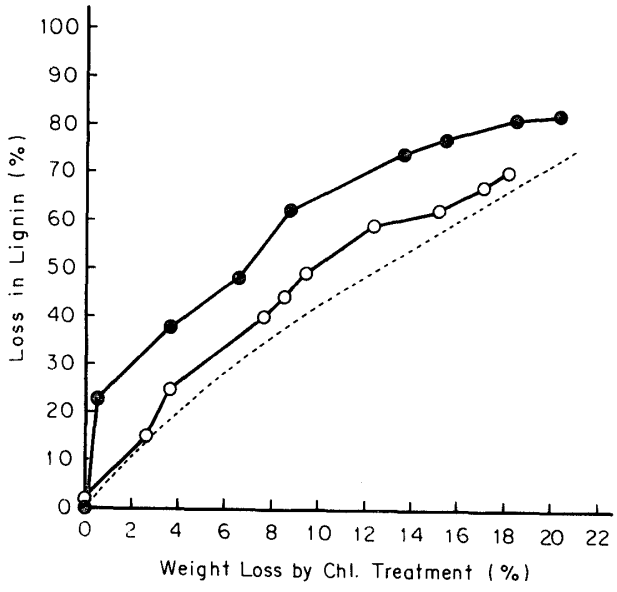

Fig. 1. Decrease of lignin in *Pinus densiflora* during chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum.* ○ *Coriolus versicolor.* Dotted line represents the pattern of lignin removal from wood shavings of *P. densiflora* during chlorite treatment[3].

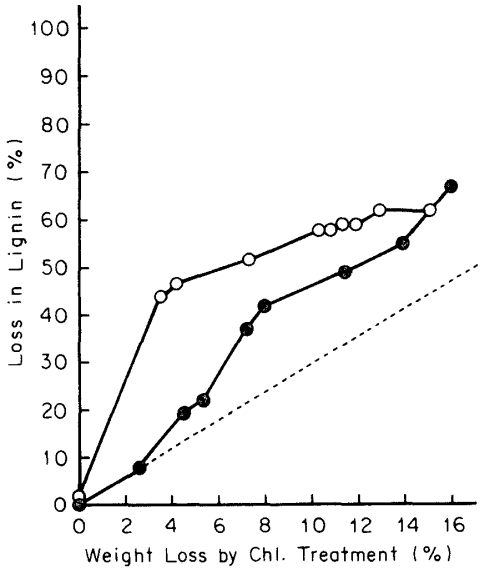

Fig. 2. Decrease of lignin in *Cryptomeria japonica* during chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum.* ○ *Coriolus versicolor.* Dotted line represents the pattern of lignin removal from wood shavings of *C. japonica* during chlorite treatment[3].

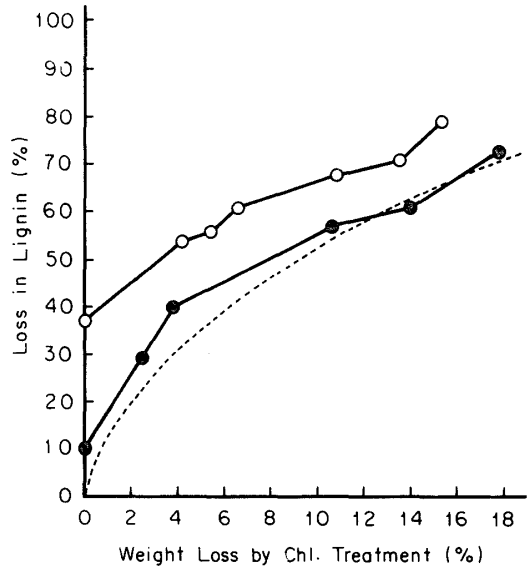

Fig. 3. Decrease of lignin in *Fagus crenata* during chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum.* ○ *Coriolus versicolor.* Dotted line represents the pattern of lignin removal from wood shavings of *F. crenata* during chlorite treatment[3].

the wood sample of *Picea mariana*, AHLGREN and GORING[8] concluded that effect of particle size was negligible in the entire range studied (0.04 to 2.0 mm thick in the longitudinal direction). On the assumption that size effect is similarly negligible or very weak between 0.3 mm thick shavings and 5.0 mm blocks used in the present experiment, difference between solid and dotted lines at a certain point on the ordinate shows rough estimate of the lignin loss caused by fungal attack.

Figs. 4, 5 and 6 show the ratio of lignin loss to weight loss in each group of the three wood species exposed to fungal attack after chlorite treatment. The ratio was calculated by dividing difference between solid and dotted lines in Figs. 1, 2 and 3 by corresponding percent loss of weight in Table 1. The ratio reaches 1.0 when the lignin and non-lignin components (assumed to be carbohydrates) are removed from wood by fungus at the same relative rates.

Figs. 7, 8 and 9 show the data on the ratio of acid-soluble lignin to total lignin

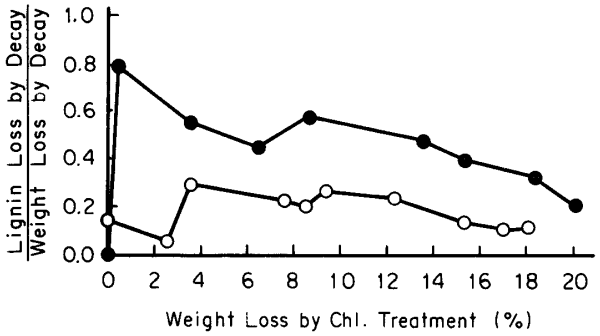

Fig. 4. The ratio of lignin loss to weight loss caused by fungal attack in chlorite treated wood of *Pinus densiflora*. ● *Chaetomium globosum*. ○ *Coriolus vesicolor*.

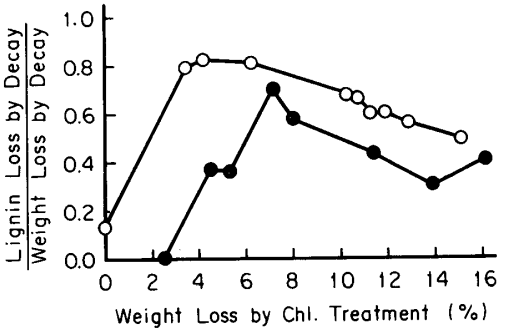

Fig. 5. The ratio of lignin loss to weight loss caused by fungal attack in chlorite treated wood of *Cryptomeria japonica*. ● *Chaetomium globosum*. ○ *Coriolus versicolor*.

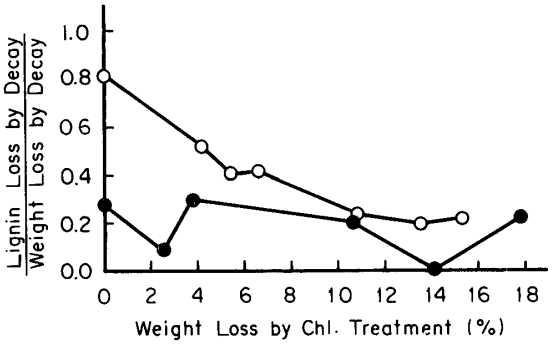

Fig. 6. The ratio of lignin loss to weight loss caused by fungal attack in chlorite treated wood *Fagus crenata*. ● *Chaetomium globosum*. ○ *Coriolus vericolor*.

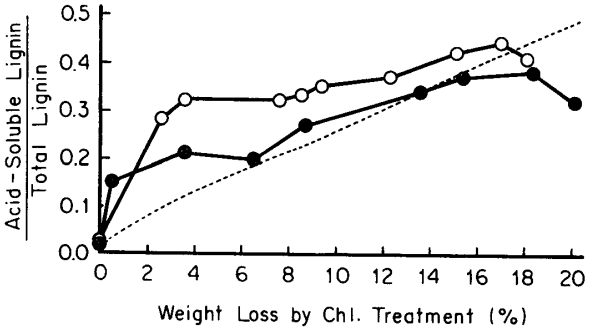

Fig. 7. The ratio of acid-soluble lignin in *Pinus densiflora* after chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum*. ○ *Coriolus versicolor*. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood shavings of *P. densiflora* during chlorite treatment[3].

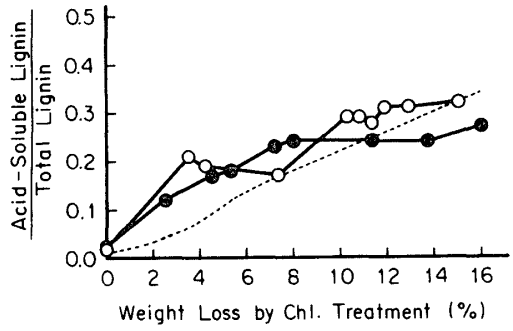

Fig. 8. The ratio of acid-soluble lignin to total lignin in *Cryptomeria japonica* after chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum*. ○ *Coriolus versicolor*. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood shavings of *C. japonica* during chlorite treatment[3].

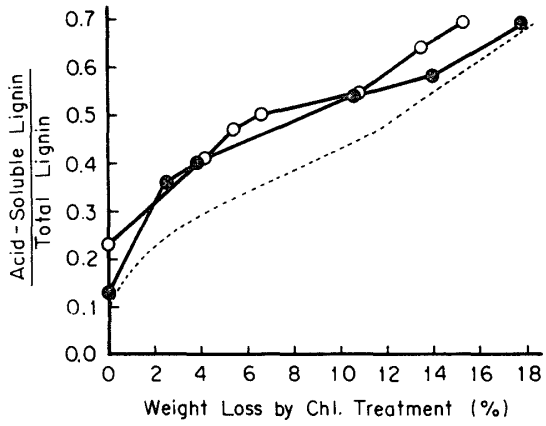

Fig. 9. The ratio of acid-soluble lignin to total lignin in *Fagus crenata* after chlorite treatment and exposure to fungal attack. ● *Chaetomium globosum*. ○ *Corivlus versicolor*. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood shavings of *F. crenata* during chlorite treatment[3].

in each group of the three wood species. The ratio was calculated by dividing percent of acid-soluble lignin by percent of total lignin. Percent of the two kinds of lignins was based on the weight of wood after decay. Dotted line represents the same ratio in wood shavings of each species after chlorite treatment[3].

A considerable amount of lignin was removed from *P. densiflora* by *Ch. globosum* at the first 0.46 % of weight loss by chlorite treatment (Fig. 1) and the ratio of lignin loss to weight loss was highest at this stage (Fig. 4). Removal of lignin from *P. densiflora* by *Co. versicolor* was slower than by *Ch. globosum* throughout all the stages, partly reflecting the lower acceleration of wood decay by the former. However, the ratio of lignin loss to weight loss for *Co. versicolor* was also smaller than for *Ch. globosum* at every stage of delignification with an exception at 0 % of weight loss (non-chlorite treatment). On the other hand, the ratio of acid-soluble lignin to total lignin for *Co. versicolor* was always larger than for *Ch. globosum* (Fig. 7), suggesting that solubilized lignin derived from insoluble Klason lignin was concentrated because of the lesser action of *Co. versicolor* for succeeding degradation.

In the case of *C. japonica*, the pattern of lignin removal was neary reverse for the two fungi. At the first 3.48 % of weight loss by delignification, large amount of lignin was removed by *Co. versicolor* (Fig. 2). The slower rate of lignin removal by *Ch. globosum* was coincident with the slower acceleration of wood decay by the fungus. The ratio of lignin loss to weight loss for *Co. versicolor* reached the maximum level at about 4 % of weight loss by delignification (Fig. 5). The ratio for *Ch. globosum* reached maximum level at about 7 % of weight loss, but was always smaller

than for *Co. versicolor*. The ratio of acid-soluble lignin for *Ch. globosum* was smaller than that for *Co. versicolor* at over 10 % of weight loss, and that for non-decayed wood shavings at greater extent of weight loss (Figs. 7 and 8). This suggests that the acid-soluble lignin was rapidly depleted by *Ch. globosum* at these stages.

Although a considerable amount of substances was removed from non-delignified softwoods by *Co. versicolor* (Table 1), no removal of lignin was detected at this stage (Figs. 1 and 2). As shown in Figs. 7 and 8, the ratio of acid-soluble lignin for *Co. versicolor* at this stage was nearly equal to the sound wood. These results assume that the lignin ramaining in non-delignified softwoods is mostly unaltered. This suggestion extends to the cases of the two non-delignified softwoods exposed to *Ch. globosum*.

It is well known that white rot fungi derive nourishment from all the major constituents of lignified cell walls—the cellulose, hemicelluloses and lignin. However, various species of white rot fungi differ in relative rates at which they remove the major components[9]. A white rot fungus used, *Co. versicolor,* is regarded as one of a group which removes the three major components approximately simultaneously[10,11]. As shown in Table 1, Fig. 3 and Fig. 6, loss of lignin and the ratio of lignin loss to weight loss in non-delignified wood of *Fagus crenata* attacked by *Co. versicolor* apparently demonstrate that wood constituents are removed at approximately the same relative rates. On the basis of these results, such a simultaneous removal of the major constituents by *Co. versicolor* always occurs only in hardwoods, but a preferential degradation of non-lignin components sometimes occurs in non-delignified or original softwoods.

The slower rate of lignin removal and the lower ratio of lignin loss in non-delignified wood of *F. crenata* attacked by *Ch. globosum* (Figs. 3 and 6) agreed with the results obtained by SAVORY and PINION[12] and LEVI and PRESTON[13].

Soft rot fungi do not attack softwoods as rapidly or as extensively as they do hardwoods. A large number of white rot fqngi including *Co. versicolor* prefer hardwoods to softwoods[14]. Hence, it is possible to assume that the lignin in softwoods is more or less a hindrance to both types of wood-decaying fungi. Of the two types of acceleration pattern of decay observed in the two softwoods, the rapid and shorter acceleration was accompanied with the rapid rate of lignin removal and the higher ratio of lignin loss to weight loss at first stage of delignification. In such a case, hindrance by lignin may be rather qualitative than quantitative, so that a certain modification of lignin which was caused by the chlorite treatment for a short time seems to act as a trigger for succeeding degradation of the modified lignin. On the other hand, the slow and longer acceleration of decay was accompanied with slow or poor overall removal of lignin. In the case of *P. densiflora*

attacked by *Co. versicolor,* the lignin was removed at apparently slower rate than non-lignin components, and removal or modification of lignin acts largely for facilitating to gain access to the carbohydrates. In such a case, hindrance by lignin may act rather quantitatively. In the case of *C. japonica* attacked by *Ch. globosum,* however, hindrance by lignin may be rather qualitative at least during the first 7 % of weight loss by delignification, since the ratio of lignin loss to weight loss increased in proportion to the extent of delignification and reached the maximum level at about 7 % of weight loss. This suggests that the lignin is a greater qualitative hindrance to *Ch. globosum* for *C. japonica* than for *P. densiflora.*

Although *F. crenata* is highly susceptible to both fungi, the rate of lignin removal and the ratio of lignin loss to weight loss were always slower and smaller in *Ch. globosum* than in *Co. versicolor.* If it is assumed that the lignin is also a hindrance to both fungi even in this easily attackable hardwood, removal of lignin by chlorite treatment may help both fungi for reducing the hindrance. This hindrance-reducing system may be less operative as the amount of lignin decreases. This was confirmed in *Co. versicolor* by the constant decrease in the ratio of lignin loss to weight loss but not in *Ch. globosum* (Fig. 6). In *F. crenata* attacked by *Ch. globosum,* the poor removal of lignin and the lower ratio of lignin loss to weight loss were observed throughout the stages. From the results, it can be considered that the lignin is not a hindrance to *Ch. globosum* in *F. crenata* and removal of lignin by chlorite treatment is less helpful for attack of the wood by the fungus.

The acid-soluble lignin was estimated from absorbance of the filtrate at 205 nm since degradative products of carbohydrate give only slight interference at this wave length[6]. The amount of soluble lignin can be calculated only when the absorptivity is known or assumed. For the determination of acid-soluble lignin in sound wood, the absorptivity can be determined by testing some standard lignin. However, preparations of other types or those obtained from other species may give somewhat different values. Moreover, some substances which should be properly regarded as lignin, such as modified or degraded products derived from the original lignin, may have an unknown absorptivity, and determinations of suluble lignin in such preparations based on ultraviolet absorbance can be considered only as approximations[6]. The absorptivity used in the present determination was 110 g^{-1} $l\,cm^{-1}$ for all preparations of each species. This is an average of the values of 113 and 106 g^{-1} $l\,cm^{-1}$, respectively, for birch and eucalyptus acid-soluble lignin preparations determined by SWAN[15] and was used in the determination of soluble lignin of 16 species by MUSHA and GORING[7]. Propriety of the use of the value, which should be examined, was not considered in the present report.

In the investigations on the chemical changes of wood caused by wood rot

fungi, lignin is analyzed mostly by the sulfuric acid method and determined as insoluble Klason lignin. Acid-soluble lignin is scarcely determined and mostly included among other materials than major wood components. ESLYN et al.[16] demonstrated that the other materials considerably increased in proportion to the increase of weight loss. The amount of soluble lignin is small in original sound woods (Table 1) but becomes increasingly large within a limited range of delignification and fungal attack. Soluble lignin is mostly regarded as the degraded or modified lignin caused by the chlotite treatment and fungal attack. Hence, accepting that estimation by ultraviolet absorption can not be fully accurate, estimation should be made on rather determination of total lignin (insoluble Klason lignin plus the ultraviolet-estimated acid-soluble lignin) than that of insoluble Klason lignin only to obtain the preciser amount of lignin remaining in decayed wood.

From these results obtained, it is apparent that the effect of delignification varies with wood and fungal species. These complicated results could be caused by some factors varying with wood and fungal species—chemical and topochemical natures of lignin, the nature of the lignin-carbohydrate association, topochemical effect on delignification, and fungal enzyme systems involved in breaking down of wood components.

It is well known that hardwood lignin contains both guaiacyl and syringyl residues but softwood lignin contains guaiacyl residue only. MUSHA and GORING[17] demonstrated by ultraviolet microscopy that the walls of fibres and ray cells contain mostly syringyl residue, and that the vessel walls and cell corner regions contain mostly guaiacyl residue. Recently, KIRK et al.[18] reported that *Co. versicolor* degrades syringyl-rich lignin first and then guaiacyl-rich lignin in the attack of birch wood, through the propressive action of enzymes from the lumen surfaces toward the middle lamella. However, at the present stage, it is yet uncertain whether such a successive degradation of lignin residues is related to the preferential attack of hardwoods by white rot fungi. Although softwood lignin contains guaiacyl residue only, microscopic distribution of the residue in wood tissue and the association with carbohydrates probably varies at some extent with species. In addition, chemistry of lignin degradation by soft rot fungi has not yet been studied in detail more than other wood rot fungi. To define more accurately the significance of ligin in different decay resistance of woods, a further knowledge of factors described above is needed.

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